

WHAT IS CLAIMED IS:

1. A method for producing disaggregated biologically active protein from a mixture comprising aggregated protein comprising the steps of:

- (a) adjusting total protein concentration in the mixture to from about 0.01 mg/mL to about 500 mg/mL;
- (b) adding to the mixture a chaotropic agent at a concentration of from 0 M to about 8 M, the concentration of the agent being limited to provide for retention of biological activity of the protein;
- (c) after step (b), increasing the pressure on the mixture to from about 0.25 kbar to about 12 kbar for a time and temperature sufficient for disaggregation of the protein; then
- (d) incubating the mixture under pressure in the range from about 0.25 kbar to about 3.3 kbar for a time from about 0.10 hours to about 12 hours; then
- (e) reducing the pressure to atmospheric pressure, whereby aggregated protein in the mixture is disaggregated and biological activity is retained.

2. The method of claim 1, wherein during the incubations step (d) the mixture further comprises an oxidizing agent and a reducing agent wherein the oxidizing agent is oxidized glutathione and the reducing agent is dithiothreitol.

3. The method of claim 1, wherein the pressure in the incubation step (d) is from about 0.5 kbar to about 3.3 kbar.

4. The method of claim 3, wherein during the incubation step (d) the chaotropic agent is guanidine hydrochloride present at a concentration from about 0.1 to about 1M.

5. The method of claim 4, wherein during the incubation step (d) the protein concentration is from about 1 to about 100 mg/mL.
6. The method of claim 4, wherein during the incubation step (d) the protein concentration is from about 1 to about 20 mg/mL.
7. The method of claim 1, wherein after steps (a) through (d), the concentration of the chaotropic agent, if present, is decreased to less than about 0.1 M.
8. The method of claim 1, wherein, prior to step (a), the aggregated protein is first treated with a reducing agent.
9. The method of claim 1, wherein the mixture of protein in step (a) comprises a detergent.
10. The method of claim 9, wherein the detergent is selected from the group consisting of sodium dodecyl sulfate, polyethoxysorbitan, deoxycholate, sodium octyl sulfate, sodium tetradecyl sulfate, polyoxyethylene ethers, sodium cholate, octylthioglucopyranoside, n-octylglucopyranoside, alkyltrimethylammonium bromides, alkyltrimethylammonium chlorides, sodium bis (2-ethylhexyl) sulfosuccinate.
11. A method for producing renatured, biologically active protein from a soluble denatured protein solution, said method comprising the steps of:
- (a) adjusting the concentration of denatured protein in solution to from about 0.01 mg/mL to about 500 mg/mL in the presence of a chaotropic agent in the concentration range of from about 2 M to about 8 M.
- (b) increasing pressure on the solution of denatured protein, which solution further comprises a chaotropic agent to from about 0.25 kbar to about 3.5 kbar; and

(c) incubating the solution of denatured protein under a pressure from about 0.25 kbar to about 3.3 kbar for from about 0.10 hours to about 12 hours; then

(d) reducing the chaotropic agent concentration to a level sufficient to permit biological activity of the protein at atmospheric pressure; then

(e) after step (d), reducing the pressure to atmospheric pressure,

whereby the protein has refolded to assume a native conformation and has biological activity of the native protein.

12. The method of claim 11, wherein during the incubation step (c), the solution or suspension further comprises an oxidizing agent, and a reducing agent wherein the oxidizing agent is oxidized glutathione and the reducing agent is dithiothreitol.

13. The method of claim 11, wherein the pressure in the incubation step (c) is from about 0.5 kbar to about 3.3 kbar.

14. The method of claim 13, wherein during the incubation step (c) the chaotropic agent is guanidine hydrochloride present at a concentration from about 0.1 to about 1M.

15. The method of claim 14, wherein during the incubation step (c) the protein concentration is from about 1 to about 100 mg/mL.

16. The method of claim 14, wherein during the incubation step (c) the protein concentration is from about 1 to about 20 mg/mL.

17. The method of claim 11, wherein at step (d), the concentration of the chaotropic agent is decreased to less than about 0.001M.

18. The method of claim 11, wherein, prior to step (a), the solubilized denatured protein is treated with a reducing agent.
19. The method of claim 11, wherein the solution of protein in step (a) comprises a detergent.
20. The method of claim 19, wherein the detergent is selected from the group consisting of sodium dodecyl sulfate, polyethoxysorbitan, deoxycholate, sodium octyl sulfate, sodium tetradecyl sulfate, polyoxyethylene ethers, sodium cholate, octylthioglucopyranoside, n-octylglucopyranoside, alkyltrimethylammonium bromides, alkyltrimethyl ammonium chlorides, sodium bis (2-ethylhexyl) sulfosuccinate.

add a²